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Thylakoid membrane fluidity and thermostability during the operation of the xanthophyll cycle in higher-plant chloroplasts

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Abstract

Barley leaves were exposed for several min to a white light of photon flux density 1000 μmol m^{-2} s^{-1}, leading to a massive conversion of the xanthophyll violaxanthin to antheraxanthin and zeaxanthin in the absence of lipid peroxidation. Using electron spin resonance spectroscopy and different spin-labeled stearate probes, we observed that this light treatment noticeably decreased thylakoid membrane lipid fluidity. The light-induced membrane rigidification (i) was proportional to the amount of zeaxanthin present in the membranes, (ii) was blocked by dithiothreitol, a potent inhibitor of the violaxanthin de-epoxidase, (iii) was slowly reversible in the dark, (iv) was not observed in thylakoids of an *Arabidopsis* mutant that has no xanthophyll cycle and (v) was accompanied by a substantial increase in the thermostability of the ionic permeability properties of the thylakoid membranes. The amount of xanthophyll-cycle pigments found in photosystem II was observed to significantly decrease after illumination. Photoacoustic and chlorophyll fluorometric analyses of the illuminated leaves revealed that strong illumination decreased the quantum yield of photosynthetic oxygen evolution and the pigment antenna size of photosystem II in green light (preferentially absorbed by carotenoids) but not in red light (absorbed by chlorophylls only). Taken together in the light of previous in vitro data on carotenoids incorporated into artificial membranes, our results indicate that the xanthophyll cycle could be an 'emergency mechanism' that rapidly provides thylakoid membrane lipids with rigidifying carotenoid molecules upon sudden increase in light intensity. The significance of this mechanism for the membrane function and adaptation to stressful light and temperature conditions is discussed. © 1997 Elsevier Science B.V.

Keywords: Membrane fluidity; Chloroplast; Light-harvesting chlorophyll-protein complex; Xanthophyll; Lipid peroxidation; Strong light stress

1. Introduction

Biological membranes are usually described as self-assembled bilayers of amphipathic di-acyl phospholipids in which proteins are embedded. However, terpenoids (formed out of branched C5 prenyl units) constitute another class of molecules that are universal components of membranes [1]. In fact, no terpenoid-free cellular membrane is known, strongly suggesting that terpenoids are essential for membrane

Abbreviations: Vio: Violaxanthin; Ant: Antheraxanthin; Zea: Zeaxanthin; PS: Photosystem; SASL: Stearic acid spin label; PFD: Photon flux density; LHCII: Light-harvesting chlorophyll *a*/*b*-protein complex of PSII; LHCIIb: The major LHCII; HPODE: Hydroperoxy octadecadienoic acid; HPOTE: Hydroperoxy octadecatrienoic acid; τ_c: Apparent rotational correlation time; $2T'_{\parallel}$: Maximum splitting; DTT: Dithiothreitol; DCMU: Dichlorophenyl-dimethylurea; MGDG: Monogalactosyldiacylglycerol

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function and/or structure. Evidence has accumulated that one of the main roles of membrane terpenoids is to reinforce and stabilize membrane architecture [1,2]. For instance, in most eucaryotic membranes, sterols, such as cholesterol, act as 'nails' inserted into one half of the lipid bilayer [3]. The hydroxyl group of cholesterol forms a hydrogen bound with the head group of a phospholipid and its dimensions allow cooperative van der Waals attractive forces to elongate and order the lipidic chain. In procaryotic membranes that do not contain cholesterol, this function is most probably fulfilled by other compounds of similar dimension (e.g., hopanoids) [4] or by carotenoids [5–7]. In vitro studies of artificial membranes have shown that carotenoids behave like 'rivets' spanning the entire membrane bilayer and bracing together the two halves of the bilayer [8–11].

Relatively little attention has been paid to the terpenoids that stabilize the thylakoid membranes of higher-plant chloroplasts. This is somewhat surprising because thylakoids have a unique lipidic composition characterized by high amounts of polyunsaturated galactolipids [12]. Consequently, as compared to many other biological membranes, the thylakoid membrane is a relatively fluid and thermolabile system and is a very sensitive target for photodestruction by active forms of oxygen produced at high light intensities. The thylakoid membrane contains no cholesterol or readily detectable amounts of other sterols [13]. It has been suggested that mechanical reinforcement of photosynthetic membranes could be achieved by α-tocopherol [14]. This suggestion is mainly based on the observation that α-tocopherol incorporated into liposomes influences the membrane fluidity in a manner similar to cholesterol [15,16] and that the level of α-tocopherol seems to increase in plant leaves exposed to environmental stresses which are potentially detrimental for membranes [17]. However, the rigidifying effect of α-tocopherol has never been demonstrated in native thylakoid membranes. The thylakoid membrane contains a variety of other terpenoids, including chlorophylls, carotenoids and plastoquinone, but they are bound to proteins in situ and/or are involved in specialized tasks such as light harvesting, photochemical energy conversion or electron transport in photosynthesis and photoprotection of photosynthetic pigments. However, it was recently reported that the operation of the so-called xanthophyll or violaxanthin cycle could be associated with a modification of the thylakoid membrane fluidity [18,19]. The xanthophyll violaxanthin (Vio) differs from the other carotenoid pigments of higher-plant chloroplasts with respect to its localization and response to rapid changes in the plant's light environment. Vio is weakly bound to the periphery of the chlorophyll antennae of the photosystems [20,21] where it acts as an accessory pigment and it presumably plays a structural role [22]. Under a high transthylakoid pH gradient (e.g., in strong light that is saturating for photosynthesis), Vio undergoes a rapid transformation to zeaxanthin (Zea) via antheraxanthin (Ant) by a de-epoxidizing enzyme localized in the lumen of thylakoids [23–25]. Vio is regenerated by light-independent epoxidation of Zea. The phototransformation of Vio in the xanthophyll cycle is correlated with changes in the structure/organization of the light-harvesting chlorophyll-protein complexes (LHCII) of photosystem II (PSII) which are revealed by an increased rate of thermal energy dissipation [24,26]. In addition, high light irradiances have been reported to bring about a restriction of the motional freedom of spin labeled fatty acids incorporated in thylakoid membranes [18]. Recently, we have shown that the conversion of Vio to Zea in dark-adapted leaves infiltrated with ascorbate at pH 5 is accompanied with an increased thermostability of the thylakoid membrane [27]. It was also observed that the operation of the xanthophyll cycle was associated with the maintenance of a low level of lipid peroxidation [28]. Observations of this nature suggest that the Zea molecules synthesized from Vio in the xanthophyll cycle interact with the lipid phase of the thylakoid membranes. This has led to the hypothesis that one of the functions of the xanthophyll cycle could be to transiently provide thylakoid membrane lipids with an efficient stabilizer–protector under potentially harmful light and temperature conditions [29,30]. The work presented in this report was undertaken to check this hypothesis. Electron spin resonance (ESR) spectroscopy and flash kinetic spectrophotometry were used to measure the fluidity and the ionic permeability of thylakoid membranes in leaves exposed to various light and/or temperature treatments. We also examined the carotenoid composition of the light-harvesting complexes of PSII and the photochemical activity of leaves in (red or green)

light preferentially absorbed by carotenoids or chlorophylls. We obtained data suggesting that Zea synthesized in strong light is released from the light-harvesting complexes of PSII in the thylakoid membrane lipid phase and we found that Zea lowered membrane lipid fluidity and enhanced membrane thermostability.

2. Materials and methods

2.1. Plant material and treatments

Unless specified otherwise, barley plants (*Hordeum vulgare* L., cv. Plaisant) were grown under controlled conditions of temperature (25/20°C), light (350 μmol m^{-2} s^{-1}, 12 h per day) and air humidity (65%). The experiments were performed on the second leaf of 3-week-old plants. The wild type (ecotype Landsberg *erecta*) and the *aba-1* mutant of *Arabidopsis thaliana* (L.) Heynh. were grown in a high humidity atmosphere at 22/17°C (day/night) and at 500 μmol m^{-2} s^{-1} (8 h per day) in a phytotron. *Arabidopsis* leaves were used before stem elongation.

The violaxanthin-to-zeaxanthin conversion was induced by exposing detached leaves (placed on a wet filter paper) to a rather strong white light of photon flux density (PFD) 1000 μmol m^{-2} s^{-1} (unless specified otherwise) at 23°C, as previously described [28]. Light was produced by metal halide lamps (Osram) equipped with three infra-red suppressor filters (Schott). Leaf temperature was maintained constant at 23°C and was monitored with a tiny PT-100 thermistance. Leaf infiltration with dithiothreitol (DTT) was done by keeping cut leaves at a PFD of 40 μmol m^{-2} s^{-1} with their petiole in a solution of 3 or 5 mM DTT or in distilled water (controls) for 3 h. Heat stress was imposed on detached leaves by exposing them to a given temperature (from 30 to 40°C) in darkness for 15 min, as previously described [27]. Photon flux densities were measured with a Li-Cor quantum meter (Li-185B/Li-190SB).

2.2. Thylakoids, LHCII and PSII-core preparations

Thylakoid membranes were prepared according to Robinson and Yocum [31] except that 5 mM DTT was added to the isolation medium and that Tricine was replaced by HEPES in the last extraction step. The major light-harvesting complex of PSII (LHCIIb) was purified using the Mg^{2+} precipitation method of Krupa et al. [32]. Thylakoid membranes and purified LHCIIb were stored at −80°C respectively in 0.4 M sucrose, 20 mM HEPES pH 8, 15 mM NaCl, 3 mM $MgCl_2$ and in 20 mM Tricine–NaOH pH 8, 2 mM EDTA, 200 mM sorbitol before further analysis. The core complex and the light-harvesting complexes of PSII were also isolated from PSII-enriched membranes by solubilization in 0.4% dodecyl β-D-maltoside and sucrose-gradient ultracentrifugation following the protocol of Bassi and Dainese [33]. The green bands corresponding to the minor and major light-harvesting complexes of PSII (B2 + B3 + B4 = LHCIIb + CP26 + CP29 + CP24) and to the PSII core (B5 + B6 + B7) were harvested with a syringe and analyzed for their pigment content.

2.3. Pigment determinations

Pigments from leaves, pelleted thylakoids, LHCIIs or PSII core complexes were extracted in methanol. After centrifugation and filtration, the samples were analyzed by reversed-phase HPLC, using a procedure adapted from the method of Gilmore and Yamamoto [34]. Pigment separation was performed at room temperature on a 5 μm Spherisorb ODS1 column (Alltech, USA) at a flow rate of 1.2 ml min^{-1}, with a 20 min gradient from 5 to 40% of solvent A (methanol–ethyl acetate, 68:32, v:v) in solvent B (acetonitrile–methanol–water, 72:8:3, v:v:v), followed by return to the initial solvent conditions. Pigment concentrations were calculated using standards and published extinction coefficients. Purified zeaxanthin and lutein were obtained from Extrasynthèse (Genay, France). The other carotenoids, chlorophyll *a* and chlorophyll *b* were prepared by TLC with n-hexane–isopropanol (100:10, v:v) as solvent system.

2.4. Quantification of hydroxyperoxy fatty acids

Hydroperoxy octadecadienoic acid (HPODE) and hydroperoxy octadecatrienoic acid (HPOTE) contents of barley leaves were determined by quantification of their corresponding hydroxy derivatives (HODE, HOTE). Both sample preparation and analysis were

conducted according to the protocol of Degousée et al. [35] except that HPLC was performed at a flow rate of 1.5 ml min^{-1} on a Waters HPLC system equipped with a Zorbax Rx-Sil column (Rockland).

2.5. Electron spin resonance (ESR) spectroscopy

Thylakoid membranes were washed twice, first with 100 mM sorbitol, 5 mM EDTA, 20 mM Tricine at pH 8, then with 100 mM sorbitol. The resulting unstacked membranes were finally resuspended in Tris 0.1 M pH 8.5 at a chlorophyll concentration of 1 mg ml^{-1}. A 100 μl sample of this preparation was vigorously mixed with 2 μl of a methanolic solution of the spin label (2 mg ml^{-1}). The following spin labels (Sigma) were used: 5-, 7-, 12- and 16-doxylstearic acid (5-SASL, 7-SASL, 12-SASL, 16-SASL). ESR experiments with *x*-SASL are performed in basic media to ensure spin probe ionization, usually at pH of around 7.5–8 for biological membranes (see e.g. [36–41]). Higher pH values (up to 9.5) can be used for model membranes but are not suitable for biological membranes because those pH are non-physiological. In this study, the pH of the suspension medium was 8.5, which is a kind of compromise between more physiological pH and extent of ionization of spin probes. Indeed, we observed that the PSII photochemical efficiency (F_v/F_m) of isolated thylakoids remained stable (ca. 0.73) at pH 8.5 but rapidly fell to 0.55 at pH 9.5 indicating a strong inhibition of the photochemical activity. After centrifugation, free spin label could not be detected in the supernatant. The labeled membrane suspension was rapidly introduced into a 50 μl glass disposable pipette (Corning) sealed at one end. Paramagnetic resonance signals of the samples were recorded with a Bruker ESP 300 (9.5 GHz) spectrometer equipped with a temperature control unit cooled with liquid nitrogen (Bruker, Karlsruhe, Germany). Typical ESR parameter settings were: Magnetic-field strength, 3390 G; microwave power, 20 mW; modulation frequency, 100 kHz; scan range, 100 G; modulation amplitude, 1.0 G; usual scan time, 6–8 min (4 to 5 scans); gain, 2×10^5. The rotational correlation time (τ_c) was calculated from: $\tau_c = 6.5 \times 10^{-10} W_0[(h_0/h_{-1})^{1/2} - 1]$ where W_0 is the width of the mid-field line and h_0 and h_{-1} are the heights of the mid- and highfield lines, respectively [40,41]. The maximum splitting value $2T'_{\parallel}$ was measured for 5-SASL and 7-SASL according to Ref. [18], when the τ_c calculation was not applicable (i.e., when τ_c was close or above 10 ns).

2.6. Photoacoustic measurements of photosynthetic oxygen evolution

Leaf discs of 1 cm diameter were placed in the hermetically closed cell of a custom-built photoacoustic apparatus that has previously been described [42]. The signal-to-noise ratio was improved by preamplifying the signal from the microphone by a SR560 low-noise preamplifier (Stanford Research Systems). The samples were illuminated with a (white, green or red) light modulated at 17 Hz. The green and red light beams were obtained by placing an Oriel 57550 broadband interference filter (peak wavelength, 510 nm; bandwidth, 60 nm; PFD at the leaf surface, 40 μmol m^{-2} s^{-1}), an Oriel 57610 filter (peak wavelength, 650 nm; bandwidth, 70 nm; PFD, 70 μmol m^{-2} s^{-1}) or a Pomfret interference filter (640 nm or 680 nm; PFD, 18 or 11 μmol m^{-2} s^{-1}, respectively) in front of the modulated light source. The amplitudes (Apt) of the photothermal signals measured with the green and red light beams in the presence of a strong (photosynthetically saturating) background light (> 4000 μmol m^{-2} s^{-1}) were adjusted to the same level (by placing neutral density filters) so that approximately the same number of light quanta was absorbed by the leaf samples in the green and red spectral regions (510 and 650 nm). The modulated oxygen-evolution component of the photoacoustic signal (amplitude, Aox) was separated from the photothermal component using the light saturation and phase adjustment method developed by Poulet et al. [43]. Photochemical energy storage was estimated at a high modulation frequency of 460 Hz. The quantum yield Φ of oxygen evolution (in relative values) was calculated as Aox/Apt [43].

2.7. Chlorophyll-fluorescence measurements

Chlorophyll fluorescence emission was measured at wavelengths higher than 700 nm using a Walz fluorometer (Model 101-102-103). The chlorophyll-fluorescence signals were recorded by a computer and were analyzed using the DA-100 software (Walz).

The initial level (F_0) of chlorophyll fluorescence was excited by a dim red light (650 nm) pulsed at 1.6 kHz. The maximal quantum yield of PSII photochemistry was calculated as $(F_m - F_0)/F_m$ where F_m is the maximal level of chlorophyll fluorescence obtained with a 800 ms pulse of saturating white light (5000 μmol m^{-2} s^{-1}). Leaves were infiltrated with 50 μM DCMU (dichlorophenyl–dimethyurea) in darkness for 30 min. The half time of the induced fluorescence rise in DCMU-poisoned leaves was taken as a measure of the functional antenna size of PSII [44]. The half time was measured in red light or in green light produced by a red or blue light-emitting diode combined with an Oriel 57610 filter or an Oriel 57550 filter. The PFD of the resulting light beams was 4.5 μmol m^{-2} s^{-1} (for the red light) and 2 μmol m^{-2} s^{-1} (for the green light).

2.8. Flash-induced electrochromic shift

Flash-induced electrochromic absorption band shift was measured at 515 nm in intact leaves using a laboratory-built Joliot-type kinetic spectrophotometer that has been previously described [27]. In brief, the instrument uses differential optics and a series of relatively intense measuring light pulses (each about 2 μs in duration) from a xenon flashlamp to measure the absorbance of two similar areas of the leaf. The light from these flashes is divided evenly into reference and sample beams by a specially constructed fiber optics bundle. Wavelength selection for the measuring pulses is provided by an interference filter (in this study, we used a Corion P1-515-F filter with a band width of 1 nm). The transmitted light is measured by photodiodes placed on the other side of the leaf. One of the leaf area is illuminated by a red actinic flash (provided by a high-power xenon flash lamp combined with a RG650 Schott filter) and the measurement of the absorbance change thus created is achieved by measuring the difference in absorbance between the sample (excited) and the reference (dark) areas of the leaf. To this end, the actinic flash is followed by a series of measuring flashes at user-selected intervals. The photodiodes were protected from the actinic light by blue-green color filters (Oriel 51720). The instrument is controlled by a computer which also acquired, processed, displayed and stored the data using a software written inhouse. Absorbance measurements were performed on dark-adapted samples (min. 20 min) with their ATPases in the deactivated state.

3. Results

3.1. Thylakoid membrane lipid fluidity

Various doxyl-stearic acid probes carrying their spin label at different positions on their acyl chain (5-, 7-, 12- and 16-SASL) were incorporated into isolated thylakoid membranes and their mobility was examined using ESR spectroscopy. The *x*-SASL probes used in the present study are commonly used to monitor lipid fluidity in artificial and biological membranes including mitochondrial and chloroplastic membranes (e.g., 18,36–38,40). When SASL undergoes relatively isotropic motion in a biological membrane, a 3-lines ESR spectrum is obtained, from which an apparent rotational correlation time, τ_c, can be derived as explained in Section 2. Greater freedom of motion of the spin-labeled fatty acids in the thylakoid membrane, indicating higher fluidity, is associated with a smaller value of τ_c. The limitations of this approximate motional parameter are well documented but τ_c can be considered to be a valuable indicator of the spin label mobility in the membrane lipid bilayer when its value is lower than 10 ns [40,41]. However, one has to remember that the calculated τ_c gives a real value only in the range 0.01–3 ns. Therefore, in the present study, τ_c values in the range 3–6 ns are used as qualitative indicators of lipid fluidity changes. Fig. 1 shows the temperature dependence of τ_c for 12-SASL incorporated into thylakoid membranes prepared from control (dark-adapted) barley leaves (squares). The expected decrease in τ_c with increasing temperature from 8 to 28°C showed no discontinuities (which could indicate breakdown of the approximations made in calculating correlation times) and the calculated τ_c values were smaller than 10 ns over the whole range of temperatures investigated. This basic experiment confirms the usefulness of the τ_c parameter (of 12-SASL) as a qualitative indicator of barley thylakoid membrane fluidity in our working conditions.

ESR measurements were also performed on thylakoid membranes prepared from barley leaves previously illuminated for a rather short time (10 min)

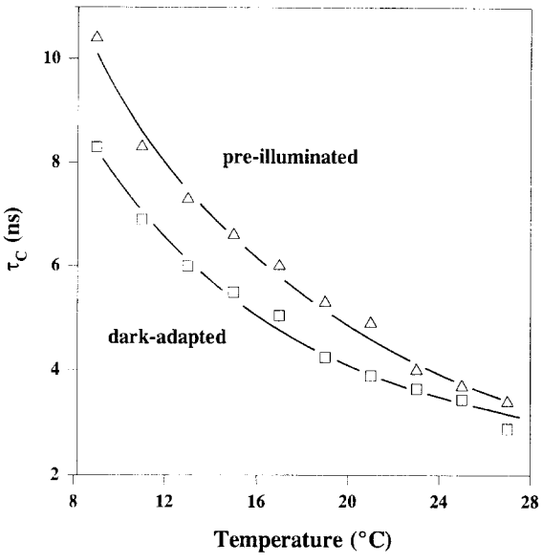

Fig. 1. ESR measurements of the thylakoid membrane fluidity at different temperatures. The plot shows the temperature dependence of the rotational correlation time (τ_c) of 12-SASL incorporated into thylakoid membranes isolated from barley leaves pre-adapted to darkness (squares) or pre-illuminated for 15 min with a white light of 1000 μmol m^{-2} s^{-1} (triangles). The $(A+Z)/(A+Z+V)$ ratio was 0.1 in thylakoids from dark-adapted leaves and 0.6 in thylakoids from light-treated leaves.

with a strong white light of 1000 μmol m^{-2} s^{-1} which led to the transformation of a large part (ca. 60%) of Vio into Zea. At a given temperature, τ_c of 12-SASL in thylakoid membranes from light-treated leaves (triangles in Fig. 1) was significantly higher than τ_c measured in dark-adapted thylakoid membranes. Thus, a short light treatment which induced the synthesis of Zea was associated with a decrease in thylakoid membrane lipid fluidity as probed with 12-SASL. 5-SASL, 7-SASL and 16-SASL were also used to monitor the lipid fluidity at different depths of the thylakoid membrane from the hydrocarbon region just beneath the phospholipid head-group (5-SASL) to the membrane center (16-SASL) (Table 1). The ESR spectra obtained with the different SASL probes had different shapes, indicating that the SASL homologs were in environments of different fluidity. However, the τ_c values of 5-SASL and 7-SASL at 13°C were not acceptable (> 10 ns). Consequently, the maximum splitting value $2T'_{\parallel}$ was calculated for those probes: $2T'_{\parallel}$ is an empirical parameter related to an order parameter of the alkyl chain motion of the spin label in a lipid core and is accepted as reflecting the fluidity of a membrane [18]. Light-induced decrease in membrane fluidity was observed with the four SASL probes using τ_c or $2T'_{\parallel}$.

In Fig. 2, we have compared the changes in τ_c of 12-SASL induced by different treatments which resulted in different levels of Zea and Ant in the thylakoid membranes. Exposing leaves for 10 min to two different PFDs (700 and 1000 μmol m^{-2} s^{-1}) resulted in an increase in τ_c which appeared to be proportional to the amounts of Zea and Ant present in the thylakoids ($(A+Z)/(A+Z+V) = 0.45$ and 0.6, respectively). Following 10 min exposure to 1000 μmol m^{-2} s^{-1}, plants were transferred back to darkness for 10 min or 3 h. The correlation time τ_c decreased back to the initial value (measured before the light treatment) after 3 h dark-adaptation but not after a short adaptation of 10 min. Again, the reversal of the light-induced change in τ_c was correlated with

Table 1
Effect of a light pre-treatment on the thylakoid membrane lipid fluidity measured by ESR and different SASL probes

	τ_c (ns)				$2T'_{\parallel}$ (G)	
	13°C		23°C		13°C	
	Dark	Light	Dark	Light	Dark	Light
5-SASL	—	—	5.5 ± 0.4	6.1 ± 0.2	50.1 ± 0.2	51.2 ± 0.5
7-SASL	—	—	5.0 ± 0.3	6.0 ± 0.1	50.2 ± 0.2	51.5 ± 0.5
12-SASL	4.1 ± 0.2	5.1 ± 0.1	3.2 ± 0.1	3.8 ± 0.2	—	—
16-SASL	1.8 ± 0.1	2.1 ± 0.1	1.3 ± 0.0	1.4 ± 0.0	—	—

Thylakoid membrane viscosity was estimated by the rotational correlation time τ_c or the maximum splitting $2T'_{\parallel}$ of *x*-SASL incorporated in thylakoid membranes isolated from dark-adapted barley leaves and from light-treated leaves (10 min at 1000 μmol m^{-2} s^{-1}). Measurements were performed at two different temperatures (13 and 23°C). Data are mean values of 5 separate experiments ± SD.

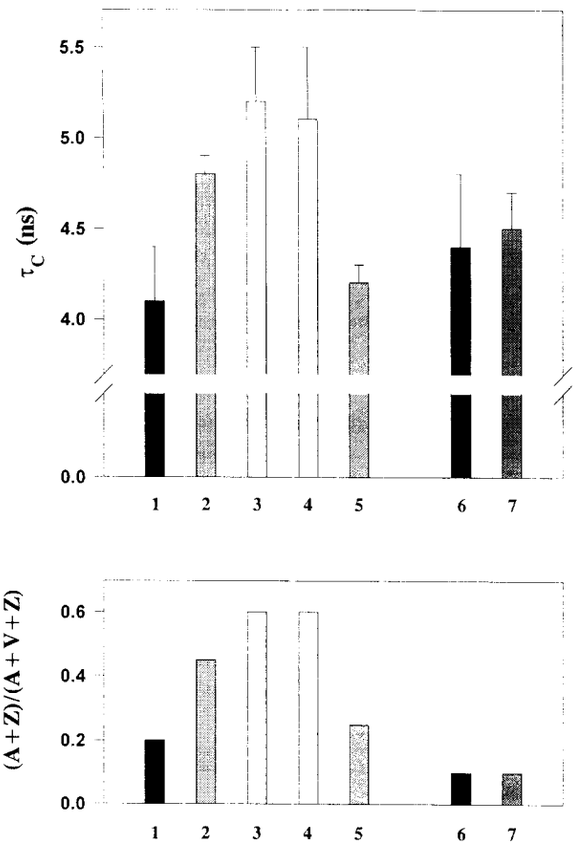

Fig. 2. Correlation between the light-induced lowering of the thylakoid membrane lipid fluidity and the amount of Zea and Ant present in the thylakoids. The rotational correlation time (τ_c) of 12-SASL at 13°C (upper panel) and the $(A+Z)/(A+Z+V)$ ratio (lower panel) were measured in thylakoid membranes isolated from barley leaves submitted to various treatments: (1) dark-adaptation; (2) 10 min exposure to a white light of 700 μmol m^{-2} s^{-1}; (3) 10 min exposure to a white light of 1000 μmol m^{-2} s^{-1}; (4) same treatment as (3) followed by 15 min dark-adaptation; (5) same treatment as (3) followed by 3 h dark-adaptation; (6) 3 h infiltration with 3 mM DTT and (7) same treatment as (6) followed by 10 min exposure to white light of 1000 μmol m^{-2} s^{-1} at 23°C. Data are mean values of 5 separate experiments. Vertical bars indicate SD.

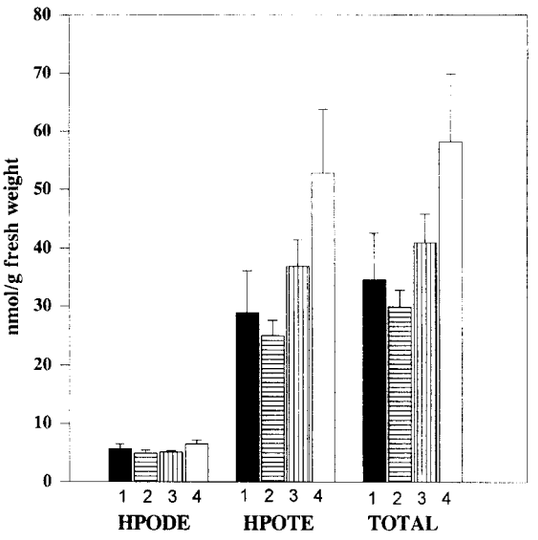

Fig. 3. Lipid peroxidation in illuminated barley leaves. The hydroperoxy fatty acid levels (HPODE and HPOTE, in nmol/g fresh weight) were measured in barley leaves before (treatment 1) and after exposure to a strong white light of 1000 μmol m^{-2} s^{-1} for 15 min (2), 30 min (3) and 2 h (4). Data are mean values of 3-to-5 separate experiments ± SD.

the amount of Zea reconverted to Vio. When leaves were infiltrated with DTT (which blocked the xanthophyll cycle), the τ_c value was slightly increased and did not change during illumination with the strong white light. Comparison of the upper panel (τ_c) and the lower panel ($(A+Z)/(A+Z+V)$) of Fig. 2 shows a clear correlation between the light-induced decrease in thylakoid membrane fluidity and the operation of the xanthophyll cycle.

It was important to check that the light treatment was mild enough not to induce any lipid peroxidation which could have caused structural perturbation of the lipid bilayer. Fig. 3 shows the hydroperoxides level of barley leaves exposed to a strong white light

Table 2
Effect of a light pre-treatment on the thylakoid membrane fluidity of wild type and *aba-1* mutant of *Arabidopsis thaliana*

	τ_c			$(A+Z)/(A+Z+V)$		
	Dark	Light	Light/Dark	Dark	Light	Light/Dark
Wild type	4.6 ± 0.3	5.8 ± 0.4	1.26	0.13 ± 0.01	0.34 ± 0.01	2.61
Mutant	3.5 ± 0.3	3.3 ± 0.1	0.94	1	1	1

Thylakoid membrane fluidity was estimated in dark-adapted ('Dark') and light-treated leaves ('Light', 1400 μmol m^{-2} s^{-1} for 10 min) by the rotational correlation time (τ_c) of 16-SASL measured at 13°C. Data are mean values of 6 (wild type) or 4 (mutant) experiments ± SD.

of PFD 1000 μmol m^{-2} s^{-1} for 10, 30 min or 2 h. A significant increase in the concentration of hydroperoxides was observed only after the longest treatment; no lipid peroxidation occurred in leaves illuminated for 30 min or less.

The light-induced decrease in thylakoid membrane fluidity was also observed in *Arabidopsis* leaves (Table 2): τ_c of 16-SASL significantly increased after 10 min at 1400 μmol m^{-2} s^{-1}. *Arabidopsis* membranes appeared to be significantly less fluid than barley membranes so that we had to measure τ_c with 16-SASL instead of 12-SASL. The *aba-1 Arabidopsis* mutant is unable to epoxidize Zea and, consequently, leaves of this mutant have no xanthophyll cycle and lack the epoxy-xanthophylls Ant, Vio and neoxanthin [45]. In a previous work [46], we showed that Zea replaced Vio, Ant and neoxanthin in the PSII light-harvesting antennae of *aba-1* chloroplasts. Furthermore, the *aba-1* mutant and the wild type could not be distinguished on the basis of their photosynthetic performances in light-limiting and light-saturating conditions [46]. Photoacoustic measurements of the oxygen evolution quantum yield Φ in green light (ca. 510 nm, absorbed by chlorophylls and carotenoids) and in red light (ca. 650 nm, absorbed by chlorophylls only) (see below for a more complete description of this type of experiments) confirmed that Zea functions as an accessory pigment in the light-harvesting complexes of the photosystems: the Φ_{510}/Φ_{650} ratio was identical in the mutant and the wild type (0.61 ± 0.03 vs. 0.62 ± 0.04). Consequently, it is very unlikely that a substantial fraction of Zea exists as a free pigment pool in the lipid phase of the *aba-1* thylakoid membranes. The ESR measurements shown in Table 2 are compatible with this conclusion: the τ_c value of *aba-1* thylakoids was not higher than that measured in wild-type membranes. In reality, the *aba-1* mutation was associated with a slight decrease in τ_c, indicating an increased membrane fluidity, a phenomenon which could be due to differences in the membrane lipid/protein ratio and/or the lipid composition. In agreement with this observation, we previously reported that the thylakoid membranes from the dark-adapted *aba-1* mutant were less stable to heat stress than thylakoid membranes from the dark-adapted wild type [46]. Exposure of *aba-1* leaves to the strong white light did not change the τ_c value indicating constant fluidity of the thylakoid membrane lipids in the absence of Vio cycle (Table 2).

3.2. Thylakoid membrane thermostability

The primary charge separation induced in the reaction centers of thylakoids by a short actinic flash is followed by a vectorial transport of charges which is accompanied by uptake and release of protons on opposite sides of the thylakoid membranes vesicles [47,48]. As a result, a transmembranous electrochemical potential builds up, causing a shift in the absorption spectrum of pigments embedded in the membrane. This so-called electrochromic shift can be monitored in intact leaves by a rapid absorbance increase at 515 nm which decays at a rate that reflects the flux of ions across the membrane down the proton gradient. In dark-adapted leaves with their ATPases in the deactivated form, the breakdown of the flash-induced electric field mainly depends on passive fluxes of charge compensating ions across the

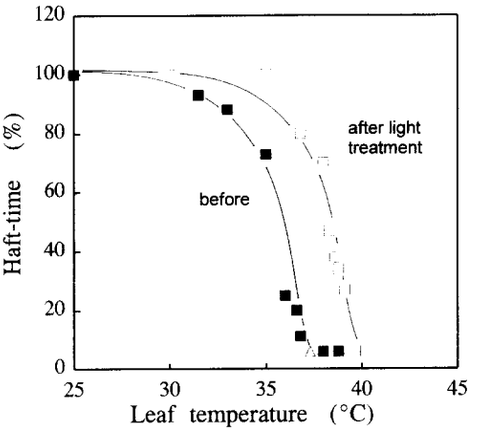

Fig. 4. Ionic permeability of the thylakoid membranes of barley leaves after heat stress. The half decay time of the flash-induced electrochromic shift was determined in barley leaves pre-exposed for 15 min to various temperatures in darkness. Closed squares: dark-adapted leaves characterized by a $(A+Z)/(A+Z+V)$ ratio of 0.12. Open squares: leaves pre-exposed for 20 min to a white light of 1000 μmol m^{-2} s^{-1} and then adapted to darkness for 20 min ($(A+Z)/(A+Z+V)=0.62$). Open triangle: leaves pre-infiltrated with 5 mM DTT and exposed to the same treatment as that described for the open squares. The electrochromic shift was measured at 25°C. The half decay time is expressed in % of the values measured before heat stress: $100\% = 216 \pm 12$ ms for the closed squares and the open triangle and 151 ± 38 ms for the open squares.

thylakoid membrane. Hence, the rate of the decay indicates the ionic permeability of the thylakoid membranes and provides a sensitive indicator of membrane intactness. Fig. 4 shows the decrease in the half decay time of flash-induced electrochromic shift (i.e., the increase in membrane conductance to ions) with increasing leaf temperature. In dark-adapted leaves containing very little Zea or Ant, the temperature at which the membrane became completely leaky to ions was around 36°C. In Zea-containing leaves (preilluminated for 20 min at 1000 μmol m^{-2} s^{-1} followed by 20 min dark-adaptation in order to deactivate the ATPases), this temperature was significantly shifted by +3°C, indicating increased thermostability of the thylakoid membranes. When the Vio cycle was blocked by DTT, light-treated leaves behaved like dark-adapted leaves. Consequently, the light-induced enhancement of membrane stability to heat stress can be related to the operation of the xanthophyll cycle.

3.3. Xanthophyll content of LHCII

The carotenoid content of thylakoid membranes isolated from dark-adapted and light-treated barley leaves was determined by HPLC and was compared with that of the major light-harvesting antenna of PSII (LHCIIb) purified from those thylakoid membranes by Mg^{2+} precipitation (Fig. 5). LHCIIb is known to bind approximately 50% of the PSII chlorophyll. Consequently, the xanthophyll-cycle pigments are mainly found in LHCIIb and not in the minor LHCIIs despite the fact that the Vio-to-chlorophyll ratio of the latter complexes is higher than that of LHCIIb [49–51]. As compared to thylakoids, LHCIIb was strongly depleted in β-carotene, contained roughly similar amounts of Vio and was enriched in lutein, neoxanthin and chlorophyll *b*, with the chlorophyll *a*/chlorophyll *b* being 1.1 ± 0.2 (vs. 2.4 ± 0.1 in thylakoids). In dark-adapted leaves, thylakoid membranes contained high amounts of Vio and no or very little Zea and Ant, as expected. In thylakoids prepared from light-treated leaves, Vio partially disappeared and was replaced by Ant and Zea. Interestingly, little zeaxanthin was found in LHCIIb although the Vio level was drastically decreased due to the operation of the xanthophyll cycle. This latter finding indicates that the bulk of Zea (ca. 50%) was not bound or was very weakly bound to LHCIIb. We also examined the pigment content of PSII (not only LHCIIb) by solubilizing PSII-enriched membranes with dodecyl β-D-maltoside and separating the PSII sub-fractions (the core, the LHCIIb and the minor LHCIIs) using ultracentrifugation in sucrose gradient (Table 3). The sucrose bands B2, B3 and B4 (according to the nomenclature defined by Bassi and Dainese,

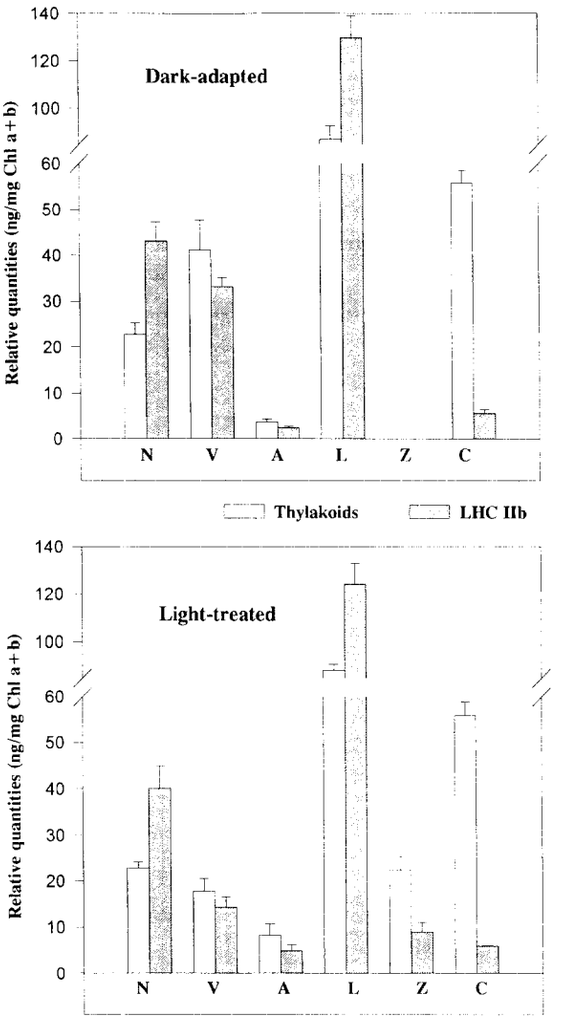

Fig. 5. Carotenoid composition of thylakoid membranes isolated from dark-adapted or light-treated barley leaves and of LHCIIb, the major light harvesting chlorophyll antenna of PSII purified from those membranes. The light treatment was 1000 μmol m^{-2} s^{-1} for 15 min. Data are mean values of at least 3 separate experiments. Vertical bars indicate SD. N = neoxanthin, V = violaxanthin, A = antheraxanthin, L = lutein, Z = zeaxanthin, C = β-carotene. Data are expressed in ng per μg total chlorophyll.

Ref. [33]) which correspond to LHCIIb and the minor LHCIIs CP24, CP26 and CP29 were collected together. It was found that approximately 50% of the Zea formed from the Vio pool during illumination did not remain in the LHCIIs. One can conclude from this finding that Zea released from LHCIIb during strong illumination did not re-bind to the minor LHCIIs. Table 3 also shows that the PSII core contains very little xanthophylls (ca. 8 ng Vio + Ant + Zea per mg chlorophyll versus 50 ng/mg in the LHCIIs) and was strongly enriched in β-carotene (around 140 ng/mg chlorophyll $a + b$). The Vio pool detected in the PSII-core bands was partially converted to Zea in the light. As previously discussed by Lee and Thornber [51], it is possible that the presence of an active Vio cycle in the PSII core was actually due to small quantities of LHCIIs which co-migrated with the core in the sucrose gradient. Again, the level of Ant and Zea found in the PSII core complexes prepared from light-treated leaves (ca. 2 ng/mg chlorophyll) was significantly lower than the level expected from the amount of Vio which was photoconverted (ca. 3.5 ng/mg chlorophyll).

The data presented in Fig. 5 and Table 3 suggest that the light-induced conversion of Vio to Zea is associated with a weaker binding of xanthophylls to LHCII. Such an effect is expected to reduce the efficiency of energy transfer from Zea to chlorophylls because carotenoid–chlorophyll singlet–singlet energy transfer requires a close contiguity of the pigments [52]. In fact, reduced energy transfer from carotenoids to chlorophylls was previously observed in pea leaves exposed to a strong light treatment [53,54]: the contribution of carotenoids to the chlorophyll fluorescence excitation spectra significantly decreased after illumination. Table 4 shows that illuminating barley leaves with a strong white light (1000 μmol m^{-2} s^{-1}) had relatively little effect on the maximal photochemical efficiency of PSII ($(F_m - F_0)/F_m$) and on the (relative) quantum yield Φ of photosynthetic oxygen evolution measured in red light with the photoacoustic method. In contrast, when measured in green light (centered at 510 nm), Φ was substantially reduced after the light treatment, with the Φ_{510}/Φ_{650} ratio falling from 0.75 to 0.55. It is important to note that the PFDs of the green and red light beams were adjusted so that the number of light quanta absorbed by the samples (indicated by the amplitude of the light-saturated photothermal signal, Ref. [43]) was similar for the red and green spectral regions. As green light is preferentially absorbed by carotenoid molecules and red light is absorbed only by chlorophylls, decreased efficiency of green light for oxygen evolution (Φ) is consistent with the existence of a light-induced excitonic uncoupling between a pool of carotenoids and the chlorophyll antennae of the photosystems [53,54]. Interestingly, no differential effect of the strong light treatment was observed between Φ measured in red light preferentially absorbed by chlorophyll a (680 nm) and Φ measured in light preferentially exciting chlorophyll b (640 nm), indicating that the observed changes in the Φ_{510}/Φ_{650} ratio did not involve uncoupling between the chlorophyll-a-containing core complex of the photosystems and the chlorophyll a/b protein

Table 3
Xanthophyll-cycle pigment content of thylakoids, of the minor and major LHCIIs and of the PSII core isolated from dark-adapted and light-treated barley leaves

	Dark-adapted				Light-treated			
	Vio	Ant	Zea	Sum	Vio	Ant	Zea	Sum
Thylakoid	58.5 ± 3.0	2.6 ± 0.2	1.5 ± 0.3	62.5	23.2 ± 1.0	5.8 ± 1.1	30.5 ± 1.5	59.5
LHCIIb + minor LHCIIs	48.1 ± 0.5	2.9 ± 0.1	0 ± 0	51.0	24.2 ± 0.5	5.4 ± 0.8	13.4 ± 0.6	43.0
PSII core	7.3 ± 0.9	0.7 ± 0.1	0 ± 0	8.0	3.8 ± 0.9	0.9 ± 0.2	1.1 ± 0.4	5.8

Violaxanthin (Vio), antheraxanthin (Ant) and zeaxanthin (Zea) (expressed in ng per μg chlorophyll $a + b$) were determined in the LHCIIs (LHCIIb + CP29 + CP24 + CP26) and the PSII core prepared by sucrose-gradient ultracentrifugation of solubilized PSII-enriched membranes. Leaves taken from barley plants grown in a greenhouse were adapted to darkness for 30 min or treated at 1000 μmol m^{-2} s^{-1} for 10 min. Data are mean values of 3 to 4 independent experiments ± SD.

Table 4
Effect of a light pre-treatment on some chlorophyll-fluorescence and oxygen-evolution characteristics of barley leaves in green or red light

	Dark	Light
$(A+Z)/(A+Z+V)$	0.13 ± 0.02(3)	0.71(2)
Oxygen evolution		
Φ_{650}	2.81 ± 0.23(3)	2.63 ± 0.19(3)
Φ_{510}/Φ_{650}	0.75 ± 0.06(6)	0.55 ± 0.04(7)
Φ_{640}/Φ_{680}	0.96 ± 0.01(3)	0.97 ± 0.01(3)
Chlorophyll fluorescence		
$(F_m - F_0)/F_m$	0.80 ± 0.01(3)	0.75 ± 0.01(3)
$t_{1/2}$ (ms)		
in red light	87 ± 10(4)	91 ± 28(5)
in green light	147 ± 16(4)	202 ± 32(5)

Leaves were illuminated for 35 min with a white light of 1000 μmol m^{-2} s^{-1} and the following parameters were measured: (i) the status of the xanthophyll cycle ($(A+Z)/(A+Z+V)$ ratio), (ii) the maximal quantum yield of PSII photochemistry $(F_m - F_0)/F_m$, (iii) the quantum yield Φ of photosynthetic oxygen evolution measured in modulated red light centered at 650 nm (Φ_{650}), (iv) the ratio Φ_{510}/Φ_{650} between Φ measured in green light centered at 510 nm and Φ measured in red light centered at 650 nm, (v) the ratio Φ_{640}/Φ_{680} between Φ measured in red light centered at 640 nm and Φ measured in red light centered at 680 nm and (vi) the functional pigment-antenna size of PSII in red light (around 650 nm) and in green light (around 510 nm) measured by the half time ($t_{1/2}$) of the induced chlorophyll fluorescence rise in the presence of 50 μM DCMU. Data are mean values ± SD. In parentheses, number of independent experiments.

complexes. Therefore, it is very improbable that the Φ_{510}/Φ_{650} changes reflect state 1-to-state 2 transitions which would have resulted in a reduction of Φ_{640}/Φ_{680}. In addition, the light-induced Φ_{510}/Φ_{650} decrease was observed to be fully reversible in darkness at a rate much slower than that of the light-state transitions (h vs. min) (data not shown).

We also estimated the functional pigment-antenna size of PSII in barley leaves by measuring chlorophyll fluorescence induction in the presence of DCMU which blocks electron flow after Q_A [44]. In accordance with the Φ measurements, 35 min exposure to 1000 μmol m^{-2} s^{-1} noticeably reduced the absorption cross-section of PSII in green light but not in red light.

4. Discussion

This work shows that strong light induces rapid and reversible changes in the physical properties of the thylakoid membranes of chloroplasts. Indeed, we found that brief exposure of leaves to a white light of PFD 1000 μmol m^{-2} s^{-1} results in a substantial decrease in thylakoid membrane fluidity, thus confirming previous observations by Gruszecki and Strzalka [18]. The light-induced change in membrane viscosity can be directly related to the operation of the xanthophyll cycle for several reasons. (i) The phenomenon was not observed when the Vio-to-Zea conversion was blocked with DTT during the light treatment (Fig. 2). (ii) The lowering of thylakoid lipid fluidity was rapidly induced (within min) and was slowly reversed in the dark (Fig. 2), as was the zeaxanthin accumulation. (iii) The extent of the fluidity change was correlated with the amounts of Vio converted to Zea and Ant (Fig. 2). (iv) The phenomenon was not observed in leaves of an *Arabidopsis* mutant having no xanthophyll cycle (Table 2). Furthermore, we can exclude that thylakoid membrane fluidity was changed due to photoperoxidative damage of lipids because no change in the hydroperoxides content of the leaves was detected after light exposure (Fig. 3). Lipid peroxidation products are known to decrease membrane fluidity [55–57]. Previously, Gruszecki and Strzalka [18] examined the fluidity and the zeaxanthin content of thylakoid membranes isolated from leaves exposed to an extreme light stress of 1400 W m^{-2} which provoked a massive destruction of lipids [53]. We confirmed in our

plant material that 30 min exposure to 1400 W m^{-2} caused an appreciable increase in the hydroperoxides level from 35.2 ± 4.5 nmol g^{-1} fresh weight to 67.8 ± 9.7 nmol per g^{-1}. Therefore, one cannot exclude that, in the Gruszecki and Strzalka's experiments, both lipid peroxidation and zeaxanthin synthesis were involved in the photoinduced changes in the motional freedom of spin labeled fatty acid probes in thylakoid membranes. Considering that the photoinduced change in τ_c was not rapidly reversed in the dark (Fig. 2), we can also exclude that the membrane fluidity alterations observed here were caused by lateral movements of protein complexes associated with the light-state transitions which are known to take place within minutes.

From experiments with artificial membranes, it is known that Zea and some other carotenoids noticeably modify the thermodynamical and mechanical properties of the membrane lipid bilayers in which they are incorporated. In particular, in vitro studies have pointed to the particular orientation of Zea in lipid bilayers with the long axis of the carotenoid being almost perpendicular to the membrane surface and the two polar end-groups anchored in the headgroup regions on both sides of the membrane [9–11]. A similar tangential alignment of the long molecular axis of zeaxanthin and lutein with respect to lipid molecules has been reported in vivo in human macula [58]. The consequences of such an orientation are a marked reduction of lipid fluidity, a general rigidification of the membrane and a decrease in membrane permeability to small molecules [9,11,59]. Evidence has also accumulated that unbound carotenoids can modulate the fluidity of bacterial cell membranes [5–7]. Taken together, those in vitro and in vivo findings have led to the idea, originally put forward by Ourisson and co-workers [1,2,4], that carotenoids could be natural regulators of the fluidity of procaryotic membranes that do not contain cholesterol. The results obtained in this study show that Zea exerts a rigidifying effect on thylakoid membranes of higher-plant chloroplasts. The zeaxanthin-related lowering of thylakoid membrane fluidity was detected with all the spin probes used here, both in the membrane center (with the 16-SASL probe) and in the peripheral region near the membrane surface (with the 5-SASL label) in accordance with the above-described transmembranous orientation of Zea in artificial lipid bilayers [9–11,59]. The scheme of Fig. 6 summarizes the results and interpretations of this study.

The special organization of the enzymes catalyzing the xanthophyll cycle, with the Vio/Ant de-epoxidase being located on the lumen-exposed side of the membrane and the Zea/Ant epoxidase being located on the other side [23,25], implies a certain freedom of movement of the xanthophyll-cycle pigments. In this context, it is worth mentioning that the purified de-epoxidase of Vio is active in vitro in the presence of monogalactosyldiacylglycerol (MGDG) only [60,61]. It has been suggested that de-epoxidation in vivo occurs at a similar lipid interface through a rapid light-dependent equilibrium between Vio in the pigment–protein complexes and a pool of MGDG [61]. In fact, energetic coupling of exogenous Vio to purified LHCIIb in the dark [62] and the desorption of native Vio from isolated LHCIIb on illumination [63] have been previously observed in vitro. The spectroscopic data of the present study (Table 4) are compatible with the existence of such a phenomenon in vivo. The existence of rapid xanthophyll exchange in vivo

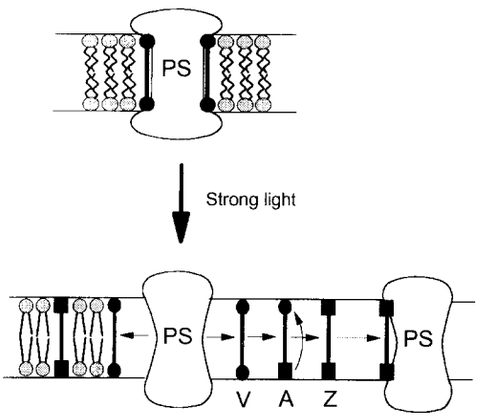

Fig. 6. Tentative model of the functioning of the xanthophyll cycle. Strong light induces conformational changes in the light-harvesting complexes of the photosystems (PS) resulting in an increased diffusional freedom of violaxanthin (V) allowing direct contact with the de-epoxidizing enzyme located on the lumen-exposed side of the membrane. Violaxanthin is transformed to antheraxanthin (A) and, possibly after a flip–flop movement, to zeaxanthin (Z) in the lipid phase of the thylakoid membrane. Zeaxanthin can subsequently re-attached to the chlorophyll antennae of the photosystems. The transient presence of xanthophylls in the membrane lipid matrix increases the viscosity, the thermostability and the resistance to photo-oxidation of the thylakoid membranes.

between the light-harvesting antennae of the photosystems and the membrane lipid phase is further supported by the finding that the amount of Zea found in the LHCIIs prepared from light-treated barley leaves is smaller than the amount of Vio really converted to Zea (Fig. 5, Table 3).

During prolonged illumination, Zea formed from Vio in the membrane lipid phase could subsequently re-attach to the light-harvesting complexes which have been suggested to bear the epoxidase activity [62] and this re-attachment could preferentially occur in some minor LHCIIs [49]. It is likely that a dynamic and complex equilibrium between bound and unbound xanthophylls will be reached during strong illumination. This can explain why the xanthophyll content of the LHCII complexes considerably varies from one study to the other: the bulk of Zea formed from Vio was found to be unattached to LHCIIb after brief (< 30 min) and/or moderate light treatments ([51,63], this study) and bound to PSII after extreme light stresses in CO_2-free nitrogen/low-oxygen mixtures [50,64]. On the other hand, release of Vio from LHCII is expected to have important repercussions on the structure-conformation of LHCII [21]. The changes in LHCII conformation that take place in high-light-treated leaves are reflected by the modifications of the yield and the excitation–emission spectra of PSII-chlorophyll fluorescence which have been attributed to a proton-induced aggregation of LHCII [26]. As pointed out by Horton and co-workers [26,65], the action of Vio as regards to chlorophyll-fluorescence quenching resembles that of a specific 'detergent' which seems to antagonize protein/protein and/or chlorophyll–chlorophyll interaction associated with chlorophyll fluorescence quenching. Thus, accumulation of a stabilizer (Zea) in the thylakoid membrane lipid bilayer and removal of a quenching inhibitor (Vio) from the light-harvesting chlorophyll antennae of the photosystems are probably two undissociable aspects of the xanthophyll-cycle activity. In this context, one has to remember that Zea differs from Vio in its polarity and in the number of carbon double bonds (11 in Zea vs. 9 in Vio). Theses differences may result in different interactions of Vio and Zea with LHCII.

The interaction of xanthophylls with the thylakoid membrane lipid phase can have important physiological implications. For instance, one can predict that Zea-induced decrease in thylakoid membrane fluidity will counterbalance heat-induced increase in the molecular motion of membrane lipids, thus protecting thylakoid membranes against disorganization. In agreement with this idea, zeaxanthin-related increase in the tolerance of thylakoids to heat stress was previously observed in ascorbate-infiltrated leaves [27] and is confirmed here under more physiological conditions (Fig. 4). Light-induced increase in the thermotolerance of the photosynthetic membranes could be very important because heat and high solar irradiances are usually combined in the field. Moreover, in bright light, thermal deactivation of excited pigments may be high enough to cause a substantial elevation of the local temperature in the lipid domain (MGDG) surrounding the photosystems. The MGDG molecules are believed to play a structural role allowing optimal packing of the large protein complexes in the thylakoid membranes [12] and the binding of lipids to some photosynthetic complexes (e.g., LHCIIb) is essential for their structural integrity [66]. Then, it is likely that local heating of the 'boundary lipids' in bright light can result in denaturation of the photosynthetic complexes. Consequently, the existence of a mechanism that rapidly provides rigidifying carotenoids to the lipid domain associated with the photosystems can protect plants exposed to intense light. In addition, the presence of free carotenoids in the thylakoid membrane lipid matrix can limit lipid peroxidation by quenching singlet oxygen [67] and scavenging free radicals [68,69]. Direct experimental support for this role has been recently provided by a study of lipid peroxidation in illuminated potato leaves [28]: Block of the Vio-to-Zea transformation by DTT or low temperature during a strong light stress resulted in pronounced lipid peroxidation whereas PSII photoinhibition was only slightly enhanced.

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References

[1] G. Ourisson, Y. Nakatani, Chem. Biol. 1 (1994) 11–23.
[2] M. Rohmer, P. Bouvier, G. Ourisson, Proc. Natl. Acad. Sci. 76 (1979) 847–851.
[3] R.A. Demel, B. De Kruyff, Biochim. Biophys. Acta 457 (1976) 109–132.
[4] G. Ourisson, M. Rohmer, K. Poralla, Annu. Rev. Microbiol. 41 (1987) 301–333.
[5] L. Huang, A. Haug, Biochim. Biophys. Acta 352 (1974) 361–370.
[6] S. Rottem, O. Markowitz, J. Bacteriol. 140 (1979) 944–948.
[7] N.R. Chamberlain, B.G. Mehrtens, Z. Xiong, F.A. Kapral, J.L. Boardman, J.I. Rearick, Infect. Immunol. 59 (1991) 4332–4337.
[8] H.Y. Yamamoto, A.D. Bangham, Biochim. Biophys. Acta 507 (1978) 119–127.
[9] T. Lazrak, A. Milon, G. Wolff, A.-M. Albrecht, M. Miehe, G. Ourisson, Y. Nakatani, Biochim. Biophys. Acta 903 (1987) 132–141.
[10] W.I. Gruszecki, J. Sielewiesiuk, Biochim. Biophys. Acta 1069 (1991) 21–26.
[11] W.K. Subczynski, E. Markowska, W.I. Gruszecki, J. Sielewiesiuk, Biochim. Biophys. Acta 1105 (1992) 97–108.
[12] K. Gounaris, J. Barber, Trends Biochem. Sci. 8 (1983) 378–381.
[13] A.A. Benson, Annu. Rev. Plant Physiol. 15 (1964) 1–16.
[14] M.J. Fryer, Plant Cell Environ. 15 (1992) 381–392.
[15] S.R. Wassall, J.L. Thewalt, L. Wong, H. Gorrissen, R.J. Cushley, Biochemistry 25 (1986) 319–326.
[16] W. Stillwell, T. Dallman, A.C. Dumaual, F.T. Crump, L.J. Jenski, Biochemistry 35 (1996) 13353–13362.
[17] A.H. Price, G.A.F. Hendry, Biochem. Soc. Trans. 17 (1989) 493–494.
[18] W.I. Gruszecki, K. Strzalka, Biochim. Biophys. Acta 1060 (1991) 310–314.
[19] M. Havaux, W.I. Gruszecki, Photochem. Photobiol. 58 (1993) 607–614.
[20] G.F. Peter, J.P. Thornber, J. Biol. Chem. 266 (1991) 16745–16754.
[21] W. Kühlbrandt, D.N. Wang, Y. Fujiyoshi, Nature 367 (1994) 614–621.
[22] F.G. Plumley, G.W. Schmidt, Proc. Natl. Acad. Sci. USA 84 (1987) 146–150.
[23] A. Hager, In: F.-C. Czygan (Ed.), Pigments in Plants, Fischer, Stuttgart, 1980, pp. 57–79.
[24] B. Demmig-Adams, W.W. Adams, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43 (1992) 599–626.
[25] E. Pfündel, E. Bilger, Photosynth. Res. 42 (1994) 89–109.
[26] P. Horton, A.V. Ruban, R.G. Walters, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 655–684.
[27] M. Havaux, F. Tardy, J. Ravenel, D. Chanu, P. Parot, Plant Cell Environ. 19 (1996) 1359–1368.
[28] J.-E. Sarry, J.-L. Montillet, Y. Sauvaire, M. Havaux, FEBS Lett. 353 (1994) 147–150.
[29] M. Havaux, F. Tardy, In: P. Mathis (Ed.), Photosynthesis: from Light to Biosphere, Kluwer, Dordrecht, 1995, pp. 777–782.
[30] W.I. Gruszecki, Acta Physiol. Plant. 17 (1995) 145–152.
[31] H.H. Robinson, C.F. Yocum, Biochim. Biophys. Acta 590 (1980) 97–106.
[32] Z. Krupa, N.P.A. Huner, J.P. Williams, E. Maissan, D.R. James, Plant Physiol. 84 (1987) 19–24.
[33] A. Bassi, P. Dainese, Eur. J. Biochem. 204 (1992) 317–326.
[34] A.M. Gilmore, H.Y. Yamamoto, J. Chromatogr. 543 (1991) 137–145.
[35] N. Degousée, C. Triantaphylidès, J.-L. Montillet, Plant Physiol. 104 (1994) 945–952.
[36] R.C. Ford, D.J. Chapman, J. Barber, J.Z. Pedersen, R.P. Cox, Biochim. Biophys. Acta 681 (1982) 145–151.
[37] R.C. Ford, J. Barber, Planta 158 (1983) 35–41.
[38] R.W. Miller, I. De La Roche, M.K. Pomeroy, Plant Physiol. 53 (1974) 426–433.
[39] D.G. McRae, J.A. Chambers, J.E. Thompson, Biochim. Biophys. Acta 810 (1985) 200–208.
[40] B. Cannon, C.F. Polnaszek, K.W. Butler, L.E.G. Eriksson, I.C.P. Smith, Arch. Biochem. Biophys. 167 (1975) 505–518.
[41] A. Keith, G. Bulfield, W. Snipes, Biophys. J. 10 (1970) 618–629.
[42] M. Havaux, F. Tardy, Planta 198 (1996) 324–333.
[43] P. Poulet, D. Cahen, S. Malkin, Biochim. Biophys. Acta 275 (1983) 433–446.
[44] S. Malkin, P.A. Armond, H.A. Mooney, D.C. Fork, Plant Physiol. 67 (1981) 570–579.
[45] C.D. Rock, J.A.D. Zeevaart, Proc. Natl. Acad. Sci. USA 88 (1991) 7496–7499.
[46] F. Tardy, M. Havaux, J. Photochem. Photobiol. B: Biol. 34 (1996) 87–94.
[47] W. Junge, Annu. Rev. Plant Physiol. 28 (1977) 503–536.
[48] H.T. Witt, Biochim. Biophys. Acta 505 (1979) 355–427.
[49] R. Bassi, B. Pineau, P. Dainese, J. Marquardt, Eur. J. Biochem. 212 (1993) 297–303.
[50] A.V. Ruban, A.J. Young, A.A. Pascal, P. Horton, Plant Physiol. 104 (1994) 227–234.
[51] A. Lee, J.P. Thornber, Plant Physiol. 107 (1995) 565–574.
[52] H.A. Frank, R.J. Cogdell, Photochem. Photobiol. 63 (1996) 257–264.
[53] M. Havaux, W.I. Gruszecki, I. Dupont, R.M. Leblanc, J. Photochem. Photobiol. B: Biol. 8 (1991) 361–370.
[54] W.I. Gruszecki, K. Veeranjaneyulu, R.M. Leblanc, Biochem. Cell. Biol. 69 (1991) 399–403.
[55] D. Hegner, Mech. Ageing Dev. 14 (1980) 101–118.
[56] J.J. Chen, B.P. Yu, Free Radic. Biol. Med. 17 (1994) 411–418.
[57] M.T. Curtis, D. Gilfor, J.L. Farber, Arch. Biochem. Biophys. 235 (1984) 644–649.
[58] R.A. Bone, J.T. Landrum, Vision Res. 24 (1984) 103–108.

[59] W.K. Subczynski, E. Markowska, J. Sielewiesiuk, Biochim. Biophys. Acta 1068 (1991) 68–72.
[60] H.Y. Yamamoto, E.E. Chenchin, D.K. Yamada, In: M. Avron (Ed.), Proc. of the Third Int. Congr. on Photosynthesis, Elsevier, Amsterdam, 1974, pp. 1999–2006.
[61] D.C. Rockholm, H.Y. Yamamoto, Plant Physiol. 110 (1996) 697–703.
[62] W.I. Gruszecki, Z. Krupa, Biochim. Biophys. Acta 1144 (1993) 97–101.
[63] W.I. Gruszecki, P. Kernen, Z. Krupa, R.J. Strasser, Biochim. Biophys. Acta 1188 (1994) 235–242.
[64] D. Philipp, A.J. Young, Photosynth. Res. 43 (1995) 273–282.
[65] D. Phillip, A.V. Ruban, P. Horton, A. Asato, A.J. Young, Proc. Natl. Acad. Sci. USA 93 (1996) 1492–1497.
[66] S. Nussberger, K. Dörr, D.N. Wang, W. Kühlbrandt, J. Mol. Biol. 234 (1993) 347–356.
[67] N.I. Krinsky, Pure Appl. Chem. 51 (1979) 649–660.
[68] G.W. Burton, K.U. Ingold, Science 224 (1984) 569–573.
[69] B.P. Lim, A. Nagao, J. Terao, K. Tanaka, T. Suzuki, K. Takama, Biochem. Biophys. Acta 1126 (1992) 178–184.